

**CLAIMS**

1. Method for detecting at least one *Mycobacterium* strain in a sample, comprising:
  - (i) providing at least one *Mycobacterium* species-specific upstream p34 gene region (us-p34) nucleotide probe,
  - (ii) reacting said us-p34 nucleotide probe with said sample under conditions that allow for the selective formation of nucleotide duplexes between said us-p34 nucleotide probe and a corresponding *Mycobacterium* nucleic acid target present in said sample, and,
  - (iii) detecting any nucleotide duplexes containing said us-p34 nucleotide probe.
- 10 2. Method according to claim 1 wherein said *Mycobacterium* species-specific us-p34 nucleotide probe specifically hybridizes with at least part of a sequence selected from SEQ ID NOs 57 to 74, or the complement thereof, or the corresponding sequences wherein T has been replaced by U.
- 15 3. Method according to claim 1 wherein said *Mycobacterium* species-specific us-p34 nucleotide probe is selected from the group of sequences represented in SEQ ID NOs 8 to 54 and SEQ ID NOs 57 to 74, or the complement thereof, or the corresponding sequences wherein T has been replaced by U.
- 20 4. Method for the differential detection of *Mycobacteria* in a sample, comprising:
  - (i) providing at least two distinct *Mycobacterium* species-specific us-p34 nucleotide probes,
  - (ii) reacting said us-p34 nucleotide probes with said sample under conditions that allow for the selective formation of nucleotide duplexes between said us-p34 specific nucleotide probe and a *Mycobacterium* nucleic acid present in said sample,
  - (iii) detecting any nucleotide duplexes containing said us-p34 nucleotide probe, and,
  - (iv) inferring from the nucleotide duplex formed, the presence and the identification of a specific *Mycobacterium* strain.
- 25 5. Method according to claim 4 wherein said *Mycobacterium* species-specific us-p34 nucleotide probes are selected from the group of sequences represented in SEQ ID NOs 8 to 54 and SEQ ID NOs 57 to 74.
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6. Method for detecting at least one *Mycobacterium* strain in a sample, comprising:

- (i) providing at least one suitable primer pair comprising a sense or antisense *Mycobacterium* species-specific us-p34 primer,
- 5 (ii) reacting said us-p34 primer pair with said sample under conditions that allow for the selective amplification of an us-p34 sequence in a *Mycobacterium* nucleic acid present in said sample, and,
- (iii) detecting the amplified product of step (ii), and,
- (iv) inferring from the amplification product the presence and the identification of at 10 least one specific *Mycobacterium* strain.

7. Method according to claim 6 wherein the sense or antisense *Mycobacterium* species-specific us-p34 primer is selected from the group of sequences represented in SEQ ID NOs 8 to 54.

15 8. Method for the differential detection of mycobacteria in a sample, comprising:

- (i) providing at least one suitable us-p34 primer pair containing a sense or anti-sense us-p34 primer,
- (ii) reacting said us-p34 primer pair with said sample under conditions that allow for 20 the selective amplification of us-p34 sequences of at least one *Mycobacterium* nucleic acid present in said sample,
- (iii) detecting the amplified product of step (ii), and,
- (iv) inferring from the amplified product formed, the presence and the identification of at least one (specific) *Mycobacterium*.

25 9. Method according to claim 8 wherein said us-p34 primer pair is selected from the group of sequences represented in SEQ ID NOs 1 to 54.

10. Method for the detection of MAC complex *Mycobacterium* species in a sample, 30 comprising:

- (i) providing at least one us-p34 probe selected from the group of sequences represented in SEQ ID NOs 8, 14, 15, 22, 27, 28, 29, 34, 35, 50, 51, 57, 68, and 73,
- (ii) reacting said us-p34 probe with said sample under conditions that allow for the 35 selective formation of nucleotide duplexes between said us-p34 nucleotide probe and a MAC complex *Mycobacterium* nucleic acid target in said sample, and,

(iii) detecting any nucleotide duplexes containing said us-p34 nucleotide probe.

11. Method for the detection of MOTT Mycobacterium species in a sample, comprising:

- 5 (i) providing at least one us-p34 probe selected from the group of sequences represented in SEQ ID NOs 9 to 13, 16 to 21, 24, 25, 26, 30 to 33, 36 to 47, 49, 53, 54, 59 to 64, 67, 69 to 72 and 74,
- 10 (ii) reacting said us-p34 primer probe said sample under conditions that allow for the selective formation of nucleotide duplexes between said us-p34 nucleotide probe and a MOTT Mycobacterium nucleic acid target in said sample, and,
- 10 (iii) detecting any nucleotide duplexes containing said us-p34 nucleotide probe.

12. Method for detecting new us-p34 sequences in a sample, comprising:

- 15 (i) providing at least one suitable primer pair comprising a sense and anti-sense us-p34 primer selected from the sequences represented in SEQ ID NOs 1 to 7,
- (ii) reacting said us-p34 primer pair with said sample under conditions that allow for the amplification of an us-p34 sequence in a Mycobacterium nucleic acid target in said sample, and,
- 15 (iii) determining the sequence of the amplification product obtained in (ii).

20 13. Method for the differential detection of mycobacteria in a sample, comprising:

- 25 (i) providing at least one suitable primer pair comprising a sense and anti-sense us-p34 primer selected from SEQ ID NOs 1 to 7,
- (ii) reacting said us-p34 primer pair with said sample under conditions that allow for the amplification of an us-p34 sequence in a Mycobacterium nucleic acid target in said sample,
- 25 (iii) selectively hybridizing the amplification products obtained in (ii) with at least one Mycobacterium species-specific us-p34 nucleotide probe selected from the group of sequences represented in SEQ ID NOs 8 to 74,
- (iv) detecting any nucleotide duplexes containing said Mycobacterium species-specific us-p34 nucleotide probe, and,
- 30 (v) inferring from the nucleotide duplex formed, the presence of a specific Mycobacterium species.

14. A *Mycobacterium* species-specific us-p34 nucleotide probe or primer comprising at least 8 contiguous nucleotides from one of the nucleic acid sequences represented in SEQ ID NOs 57 to 74, or the complement thereof, or the corresponding sequences wherein T has been replaced by U.

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15. The *Mycobacterium* species-specific us-p34 nucleotide probe or primer of claim 14 selected from the sequences as represented in SEQ ID NOs 8 to 54.

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16. A *Mycobacterium* us-p34 nucleotide primer selected from the sequences as represented in SEQ ID NOs 1 to 7.

17. A nucleic acid comprising a sequence selected from SEQ ID NOs 8 to 54, 57 to 64, 66, 67 and 69 to 74.

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18. A composition comprising at least one nucleotide probe, primer or sequence according to any of claims 14 to 17.

19. A diagnostic kit comprising a probe, primer or sequence according to any of claims 14 to 17 or a composition according to claim 18.

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20. A solid support for the detection of mycobacteria comprising fixed to said support at least two capture probes selected from SEQ ID NOs 1 to 54 and 57 to 74.

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21. A solid support according to claim 19 for use in a method of any of claims 1 to 5 or 13.

22. A method for differentiating between *Mycobacterium bovis* and *Mycobacterium tuberculosis* in a sample, comprising:

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(i) providing at least one us-p34 probe selective for *Mycobacterium bovis* or *Mycobacterium tuberculosis* wherein said probe is SEQ ID NO 66 for *Mycobacterium bovis* or SEQ ID NO 65 for *Mycobacterium tuberculosis*,

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(ii) reacting said us-p34 nucleotide probe with said sample under conditions that allow for the selective formation of nucleotide duplexes between said us-p34 nucleotide probe and a corresponding *Mycobacterium bovis* or tuberculosis nucleic acid target present in said sample, and,

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(iii) detecting any nucleotide duplexes containing said *Mycobacterium bovis* or tuberculosis specific us-p34 nucleotide probe.

23. A method for differentiating between *Mycobacterium bovis* and *Mycobacterium* tuberculosis in a sample, comprising:

- (i) providing at least one suitable primer pair comprising at least one sense or antisense us-p34 primer selective for *Mycobacterium bovis* or *Mycobacterium tuberculosis* wherein said primer is SEQ ID NO 66 for *Mycobacterium bovis* or SEQ ID NO 65 for *Mycobacterium tuberculosis*,
- 10 (ii) reacting said us-p34 primer pair with said sample under conditions that allow for the selective amplification of *Mycobacterium bovis* and/or *Mycobacterium tuberculosis* nucleic acid target present in said sample, and,
- (iii) inferring from the reaction product(s) the presence of *Mycobacterium bovis* and/or *Mycobacterium tuberculosis* in said sample.

15 24. A method for differentiating between *Mycobacterium avium* and *Mycobacterium avium* subspecies paratuberculosis in a sample, comprising:

- (i) providing at least one us-p34 probe selective for *Mycobacterium avium* and *Mycobacterium avium* subspecies paratuberculosis wherein said probe is selected from the sequences represented in SEQ ID NOS 8, 27 to 29, 50 or 58 for *Mycobacterium avium* or SEQ ID NO 68 for *Mycobacterium avium* subspecies paratuberculosis ;
- 20 (ii) reacting said us-p34 nucleotide probe with said sample under conditions that allow for the selective formation of nucleotide duplexes between said us-p34 nucleotide probe and a corresponding *Mycobacterium avium* and *Mycobacterium avium* subspecies paratuberculosis nucleic acid target present in said sample, and,
- 25 (iii) detecting any nucleotide duplexes containing said *Mycobacterium avium* and *Mycobacterium avium* subspecies paratuberculosis specific us-p34 nucleotide probe

30 25. A method for differentiating between *Mycobacterium avium* and *Mycobacterium avium* subspecies paratuberculosis in a sample, comprising:

- (i) providing at least one suitable primer pair comprising at least one sense or antisense us-p34 primer selective for *Mycobacterium avium* and *Mycobacterium*

avium subspecies paratuberculosis wherein said primer is selected from the sequences represented in SEQ ID NOs 8, 27 to 29, 50 or 58 for *Mycobacterium avium* or SEQ ID NO 68 for *Mycobacterium avium* subspecies paratuberculosis,

5 (ii) reacting said us-p34 primer pair with said sample under conditions that allow for the selective amplification of *Mycobacterium avium* and *Mycobacterium avium* subspecies paratuberculosis nucleic acid target present in said sample, and,

(iii) inferring from the reaction product(s) the presence of *Mycobacterium avium* and *Mycobacterium avium* subspecies paratuberculosis in said sample.